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The role of inflammation-related genes in osteoarthritis

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SUMMARY

In this review article we examine the role of inflammation-related genes in osteoarthritis (OA) from the perspective of genetics, epigenetics and gene expression. There have been great strides in such genomic analyses of OA in recent years thanks to the study of adequately powered patient cohorts, the detailed analysis of candidate genes, and the application of genome-wide approaches. These have led to some unexpected and therefore exciting discoveries, implicating pathways that would not necessarily have been predicted to have a role in this common arthritis. Inflammatory-related genes sit firmly in the candidate camp based on prior observations that the OA disease process can have an inflammatory component. What is clear from the genetic studies published to date is that there is no compelling evidence that DNA variation in inflammatory genes is an OA risk factor. This conclusion may of course change as ever more powerful association studies are conducted. There is, however, compelling evidence that epigenetic effects involving inflammatory genes are a component of OA and that alteration in the expression of these genes is also highly relevant to the disease process. We may in fact be close to demonstrating, at the genomic level, a clear separation of OA patients into those in whom inflammation is a key driver of the disease and those in whom it is not. This has obvious implications for the design of trials of novel OA interventions and may also guide the intelligent re-purposing of anti-inflammatory therapies.

Introduction

Over the last decade the volume of data derived from the genomic analysis of osteoarthritis (OA) has escalated almost exponentially, with the application of genome-wide association scans (GWASs), comprehensive epigenetic analyses and powerful gene expression studies. For the majority of these investigations, the OA phenotype used has been severe disease in the absence of a secondary cause, such as joint trauma, and in which the disease is so painful and debilitating that joint replacement has been undertaken. Alternative phenotypes have included injury models, in particular meniscal tears that can increase the risk of OA development. This latter model has been particularly investigated in gene expression analyses.

For a genetic association study in which *trans*-generational heritable DNA changes are being measured as opposed to somatic mutations, the cell source of the genotyped DNA is irrelevant. Blood is the tissue often used in these studies due to its relative ease of accessibility, although saliva is now a common alternative. For epigenetic and gene expression studies the source of the nucleic acid (DNA and RNA, respectively) can, however, be critical, since alterations in epigenetic status or gene expression levels can be cell or tissue-type specific with the added variability of possible temporal effects. In this regard OA has an advantage over many other common diseases in that joint replacement surgery provides researches with ready access to relevant tissues, albeit at an end stage of the disease process. Due to the central nature of cartilage in the OA disease process, the cartilage chondrocyte has been the preferred cell for many of the epigenetic and gene expression studies so far performed. The increasing realization, however, that OA is a disease of the whole joint[1] has encouraged the analysis of a wider range of joint tissues and cell types. This has the advantage that a more comprehensive approach is being undertaken in the molecular analysis of the disease but the complication that, unlike cartilage, joint tissues such as synovium, fat pad and subchondral bone contain multiple cell types, adding heterogeneity and therefore a further level of complexity to the interpretation of data derived from these tissues.

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Here we shall review results from the latest OA genetic, epigenetic and gene expression studies and, where relevant, we shall also refer to earlier studies where they help to make a point.

OA genetics and inflammation-related genes

The use in the not too distant past of underpowered case–control cohorts in OA genetic studies threw up a large number of genetic associations. When these studies involved the targeting of candidate genes, as they invariably did, several pathways emerged that looked very promising. Amongst there were a number of inflammatory genes. However, few of these associations have withstood the application of adequately powered analyses, involving thousands rather than hundreds of OA patients, combined with the coverage of the whole human genome. These agnostic GWASs have instead highlighted replicable association signals at or close to unexpected genes[2–9]. Recent examples include a hand-OA GWAS on Icelandic patients that reported an association to *ALDH1A2* (*P*-value for the association of 3.99×10^{-10}), which codes for an enzyme involved in retinoic acid synthesis[3], and hip and/or knee OA GWASs in European cohorts that reported associations to a range of genes including *CHST11* ($P = 1.64 \times 10^{-8}$), which codes for an enzyme that sulfates proteoglycan[6]. From amongst the OA association signals reported from GWAS none encompass a gene coding for a protein involved in inflammation.

A recent meta-analysis of OA European candidate gene studies confirmed that the selection of candidates in OA genetic studies is in fact an extremely fallible exercise, with only the type XI collagen gene *COL11A1* and the vascular endothelial growth factor gene *VEGF* demonstrating any semblance of association to the disease from among the 199 candidates examined[10]. However, even their association *P*-values were much less compelling than the signals that have emerged so far from the GWAS studies, with values $>1 \times 10^{-6}$. The inflammation-related genes amongst the 199 candidates studied included those coding for tumor necrosis factor and the NF-κB protein complex, as well as several coding for interleukins and their receptors and antagonists. None of these merited particular comment in that report.

This overwhelming lack of support for inflammation as a repository of OA genetic risk is tempered by one study, reported in 2010 and involving a GWAS of knee OA in Japanese patients[11]. Here an association to the HLA class II/III region was identified, with a *P*-value of 6.73×10^{-8} in the Japanese and a *P*-value of 5.10×10^{-9} in a meta-analysis of Japanese and Europeans. The European contribution to this *P*-value was, however, quite modest, whilst subsequent attempts to replicate the signal in both Asians and Europeans has proven fruitless[12,13]. It appears possible therefore that this association is restricted to the Japanese cohort that was the source of the initial discovery.

So what to conclude? Apart from this Japanese study, there is at present no compelling evidence that DNA polymorphism within or near to genes that code for proteins involved in inflammation is a risk factor for OA. That, however, is not the end of the story. The GWAS and meta-analyzed candidate studies performed to date have focused on common DNA polymorphisms, with minor allele frequencies >10%. It is therefore possible that future analyses of less common polymorphisms will uncover associations to inflammatory genes. It is also the case that the case–control sample sizes studied to date are too small to detect particularly small-effect risk alleles (odds ratios < 1.1). Since OA is known to be highly polygenic and lacks large effect loci[8] it will be the case that as more powerful OA GWAS studies are undertaken new risk alleles will be identified. It may be that inflammatory genes will be amongst these new discoveries. Finally, the analysis of alternative OA phenotypes (for example, different compartments of the knee rather than studying knee OA as a single entity) may refine the association studies and uncover association signals in inflammatory genes relevant to particular OA subsets.

At present, however, the conclusion to draw is that there is no link between OA genetic risk alleles and inflammation-related genes.

OA epigenetics and inflammation-related genes

Epigenetic modifications are a mitotically heritable means by which the cell can regulate gene expression in the absence of an alteration in the sequence of the DNA. There are three known epigenetic mechanisms: DNA CpG methylation, histone modifications and microRNAs (miRNAs). The first two alter the accessibility of the DNA within the chromatin, influencing the binding of *trans*-acting factors and thereby modulating the initiation and level of gene transcription. miRNAs regulate the stability of mRNA transcripts and therefore regulate translation rather than transcription.

Several informative review and commentary articles have been published recently on the role of epigenetics in OA[14–18]. Most OA epigenetic studies have focused on candidate loci, often those coding for catabolic regulators of cartilage homeostasis. As more high-throughput technologies have been developed, particularly the use of arrays for CpG methylation analysis, a more genome-wide approach has been adopted, akin to changes observed in genetics with the shift from candidates to GWAS. Epigenetic effects on inflammatory genes in OA have been convincingly demonstrated using both the candidate gene and genome-wide approaches, and below we shall provide examples for each of these.

Candidate studies

Altered CpG methylation of the promoter of the pro-inflammatory interleukin-1β gene *IL1B*, with concurrent changes in its expression, has been consistently demonstrated[19,20]. These experiments involved the direct study of chondrocytes from OA patients and *in vitro* analyses including the forced de-methylation of the chondrocyte DNA with the agent 5-azacytidine. The role of histone modifications in OA has been investigated by examining the effect of histone deacetylase inhibitors (HDACi) on cartilage explants and chondrocytes from OA patients. These studies have revealed that histone acetylation/deacetylation modulates cartilage catabolism genes, in particular the matrix metalloproteinases (MMPs), in response to IL1[21]. The potential utility of this discovery has been demonstrated in the destabilization of the medial meniscus (DMM) mouse OA model, with HDAC inhibition attenuating the disease process[22].

Genome-wide approaches

Most miRNA studies performed in OA have started with a microarray-based approach to agnostically assess which miRNAs are up or down regulated in the disease, followed by a more targeted analysis. A classic example of this is the discovery that miR140 expression is reduced in OA cartilage and is also down-regulated following IL-1β stimulation of chondrocytes[23]. Subsequent studies have demonstrated that other miRNAs induce or are induced by inflammatory proteins, including miRNAs 145, 127-5p and 146a[24–26].

Genome-wide CpG methylation analyses in OA have been performed using arrays that enable the investigation of up to several hundred thousand CpGs. There has so far been six such methylation analyses reported, five investigating DNA extracted from hip or knee cartilage[27–31] and one investigating DNA extracted from trabecular bone[32]. Of the five cartilage studies numerous comparisons were made, including hip vs knee, OA vs non-OA, and cartilage adjacent to the lesion vs intact cartilage distal to the lesion.

Hypo and hypermethylated CpGs were identified and gene ontology analysis performed to identify gene families and pathways demonstrating significant clustering. Four of the five studies reported inflammation-related genes as among the most significant pathways identified[28–31]. Intriguingly, two of these four studies reported that knee OA patients and hip OA patients each segregate into clusters based on the differential methylation of inflammation-related genes. These results suggest that OA patients can be stratified on the basis of the methylation status of these genes in their cartilage, implying that inflammation is relevant to the disease in some but not necessarily all OA patients. The trabecular bone study compared OA samples with those from osteoporotic patients[32]. Inflammation was not one of the pathways identified as demonstrating differential CpG methylation between the two groups.

OA gene expression and inflammation-related genes

Through the application of gene-expression microarrays, transcriptomic analyses are identifying unique expression patterns of inflammation-related genes in OA, not only in comparison with non-OA controls, but also according to the stage of disease progression, and in the study of healthy and diseased regions of the same tissue. Below we review the gene expression data with respect to these three categories.

OA-specific differences in inflammation-related gene expression

Clear differences in inflammatory gene expression have been identified in the OA joint compared to non-OA controls, with one of the most overtly inflamed tissues being the synovium. Inflammation is less pronounced in the OA synovium in comparison to rheumatoid arthritis (RA) but correlates with both pain and structural decline[33,34]. While the precise cause of synovium inflammation (synovitis) remains to be defined, gene-expression microarray analysis shows increased expression of complement effector genes and a decrease of complement inhibitors in the OA synovium compared to controls[35]. Further to this, IL-1β has been associated with many of the pathological features of OA. Although *IL1R*, encoding the IL-1 receptor, is not differentially expressed in normal compared to OA synovial fibroblasts, IL-1R is upregulated at the protein level in OA[36]. IL-1β signaling has been shown to modulate expression of 909 out of 3459 genes in primary human articular chondrocytes, including significant induction of numerous chemokines and inflammatory mediators, such as the genes *IL11* and *CCL5*[37]. This post-transcriptional upregulation of IL-1R in OA is therefore likely to cause a significant alteration in inflammatory gene expression. In line with this, comparison of OA cartilage to microscopically intact cartilage from individuals without OA identified a distinct upregulation of chemokine and cytokine genes in OA cartilage, including *IL8* and *LIF*[38].

Expression changes in inflammatory-associated genes in cartilage have been shown to display commonality between knee and hip OA when compared to non-diseased controls[39]. For example, differential expression of genes in the TREM1 signaling pathway, involved in amplification of the inflammatory response, occurs in both knee and hip OA articular cartilage when compared with controls. In addition to localized tissue-specific differences in gene expression, systemic elevation of inflammatory gene expression is also present in OA. Microarray analysis of peripheral blood leukocytes (PBLs) from OA patients and non-OA controls identified differential expression of 173 genes, including many inflammatory genes[40]. Furthermore, a subgroup of OA patients displayed increased expression of *IL1B* of >2 fold when compared with controls. Not only does this provide support for systemic inflammation in OA, but it also supports the notion that a subgroup of OA patients may exist, for whom inflammation is a key disease driver.

Early changes in inflammatory gene expression

There is an expanding body of research reporting differential inflammatory gene expression in relation to disease progression. The unique inflammatory transcriptome in early OA is of particular interest as this precedes structural joint damage and as such, may potentially provide an option for effective therapeutic intervention. The study of early OA is troublesome, given that clinical symptoms generally do not occur until cartilage destruction has already begun. Consequently, studies often focus on groups with highly increased disease risk. The Multicentre Osteoarthritis Study (MOST) longitudinal case–control study reported that meniscal tears are a key risk factor for developing radiographic OA[41]. This suggests that joint trauma can catalyze the molecular changes underpinning later OA development. Microarray analysis of patients undergoing arthroscopic menisectomy following a meniscal tear identified 266 genes to be differentially expressed in inflamed synovium compared with synovium without inflammation[42]. Chemokines and chemokine receptors, required in innate immune activation, demonstrated the highest upregulation in inflamed synovium. In particular, expression of CCL5, a C–C chemokine able to recruit innate immune cells such as lymphocytes, was found to associate with severity of inflammation, as validated by real-time PCR. CCL5 induces IL-6 expression in a concentration-dependent manner in synovial fibroblasts via interaction with the CCR5 receptor, which displayed elevated mRNA expression in OA compared to normal synovial fibroblasts[43]. As such, it is suggested that this early upregulation of inflammatory chemokine expression is pivotal in inducing cytokine expression, which may be required to instigate the processes leading to downstream OA-pathology.

The unique early chemokine signature in the damaged joint is highly dynamic. A pilot analysis of gene expression in synovial fluid from individuals who had suffered meniscal injury identified significant changes in gene expression dependent on duration of injury[44]. Molecular characterization of synovial cell pellets and cell-free supernatant, by microarray and RNA-sequencing, respectively, revealed a distinct inflammatory expression pattern according to injury duration. In cell pellets, there was increased expression of inflammatory genes in the longer injury duration group (>2 months since injurious event), with the top 5% of genes with the greatest average expression belonging to inflammatory pathways. The cell supernatant analysis identified 764 differentially expressed genes, with SLC2A9 displaying the greatest differential expression. While not directly involved in inflammatory pathways, this regulator of glucose homeostasis is induced by OA-associated inflammatory cytokines, such as IL-1β, and may have a role in chondrocyte survival[45]. This therefore indicates that even at the very earliest stage of injury, altered inflammatory gene expression may have downstream functional implications.

Analysis of synovium from patients with confirmed early-stage OA, evidenced by knee pain and cartilage abnormality with a Kellgren–Lawrence (K–L) score of <2, supports these molecular findings. In synovial membrane biopsies obtained during arthroscopic menisectomy, *TNF*, *IL1B* and *IL6* gene expression levels in both synovial membrane and synovial fluid were at comparable levels in early OA and end-stage disease synovium[46]. This again demonstrates that changes in the inflammatory gene expression profile are an early event in OA pathogenesis.

Focal differences in inflammatory gene expression: damaged vs undamaged tissue

As discussed, gene expression data has provided evidence for tissue-wide inflammatory gene dysregulation in OA. Further to this, a distinct inflammatory gene expression pattern is also apparent at

a focal level in diseased tissues. This may hold the key to establishing why specific regions of cartilage are affected in OA.

A microarray comparison of lesioned and unlesioned cartilage in individuals with knee OA found 754 genes to be differentially expressed, with an enrichment of inflammatory genes[47]. Interestingly, genes involved in the complement system, such as *C2*, were found to be upregulated in unlesioned cartilage, possibly reflecting altered responsiveness of the lesioned regions. A more powerful microarray study identified differential expression of 1717 genes in 33 lesioned and unlesioned knee-OA cartilage pairs, including many inflammatory genes[48]. *CD55*, coding for a regulator of the complement response, was one of the most differentially expressed genes. Increased gene expression was reflected by elevated protein levels, suggestive of downstream functional consequences of abnormal focal inflammatory gene regulation. This confirmed a previous in-joint microarray analysis of five patients undergoing knee arthroplasty[49]. A total of 411 differentially expressed genes were identified, yet *CD55* was one of only six genes consistently upregulated in the lesioned group, as validated by quantitative real-time PCR in five additional specimen pairs. Increased *CD55* expression in damaged tissue may initially seem as somewhat of a paradox, as it is a negative regulator of the complement system. However, loss of *Cd55* in mice ameliorates symptoms in a collagen-induced model of RA, with significant reduction in inflammatory symptoms such as paw swelling[50]. CD55 is known to function as a ligand for CD97, present on leukocytes, although the inflammatory implications of the CD55–CD97 interaction remain to be established. Given that OA presents from a genetically diverse disease population, this reproducible evidence implies core inflammatory genes to be integral to disease pathology.

In light of the fact that all tissues in the joint display pathologic changes in OA, it is to be expected that focal inflammatory gene dysregulation is not limited to cartilage. Microarray analysis of inflamed vs macroscopically normal synovium from 12 patients with knee OA identified 886 genes to be differentially expressed, with an enrichment of inflammatory genes. *TREM1* demonstrated particularly increased expression in inflamed synovium, validated by western blot analysis[51]. Earlier research comparing lesioned and unlesioned cartilage from knee OA also identified *TREM1* to be significantly upregulated in damaged cartilage[52]. More commonly associated with RA, *TREM1* expression is induced by activation of toll like receptor 4 (TLR4) in response to acute inflammation, and results in expression of pro-inflammatory cytokines including TNF-α and IL-1β[53]. Together, these data provide evidence of focal dysregulation of inflammatory genes in damaged OA tissues, with a level of commonality between different tissue types. This may indicate that early universal dysregulation of a small group of inflammatory genes in damaged tissue is essential to the global pathologic events in the progression of OA.

Genes and OA treatments

Current guidelines recommend topical non-steroidal anti-inflammatory drugs (NSAIDs) and paracetamol as a first-line treatment for OA ahead of oral NSAIDs, with the Osteoarthritis Research Society International (OARSI) recommending topical NSAID use in those who do not respond to paracetamol[54]. This reflects the risk of cardiovascular and gastrointestinal adverse events posed by NSAIDs, which is significantly greater when taken orally[55,56]. Conflicting data has been published when comparing the effectiveness of NSAIDs and paracetamol for patients with severe pain, which could be argued to be suggestive of a variable inflammatory contribution in different OA groups[57,58]. As such, further research characterizing the role of inflammation in OA may have important bearing on future treatment stratification.

The ability therefore to identify at a genetic level OA patients for whom inflammation is a core disease modulator may prove fundamental in advancing pharmacologic intervention and facilitating the formation of differential treatment pathways. This would protect patients less likely to respond well to oral anti-inflammatory treatment from the risk of significant adverse effects, while improving the risk-benefit ratio in the inflammatory subgroup, in favor of increased benefits. The risks of adverse effects from NSAIDs are also increased by extended periods of use[55,56]. Therefore, the future potential to intervene during early stages of the disease when aberrant inflammation is initiated may create the opportunity for short-term intervention, halting disease progression while minimizing adverse effects.

Conclusions

From an epigenetic and gene expression perspective, inflammation-related genes have a clear role in the OA disease process. Most epigenetic and gene expression studies performed to date have focused on cartilage or synovial tissue. Future analyses should expand the repertoire to other tissues of the synovial joint and should also increase sample sizes from the tens of patients studied so far to the hundreds. This will provide the comprehensive coverage and power needed to establish the clearest picture possible of exactly how inflammation-related genes contribute to OA initiation and development. The recent discovery of a circulating miRNA correlating with OA progression[59] and of the differential expression between OA and healthy age-matched controls of peripheral blood genes[60] highlights that a joint-centric approach to OA may also have to be rethought.

One of the most exciting finds from the epigenetic analyses is that OA patients can be stratified into those in whom inflammation-related genes are actively involved in OA and those in whom they appear not to be. This has clear relevance to the design of clinical trials for testing disease-modifying treatments and to the potential re-purposing of anti-inflammatory drugs: the right treatment for the right patient.

From a genetic perspective inflammation-related genes are not relevant to the disease, with no compelling evidence that DNA polymorphism at these genes contributes to OA susceptibility. As noted earlier, this picture may change as ever more powerful genetic association studies are performed. There is, however, a lot of catching-up to do and considering that these genes have already received quite considerable attention it is hard to imagine that they will emerge as major repositories of OA genetic risk.

Author contributions

All authors were involved in drafting the article and revising it critically for important intellectual content, and all authors approved the final version to be published.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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References

1. Loeser RF, Goldring SR, Scanzello CR, Goldring MB. Osteoarthritis: a disease of the joint as an organ. Arthritis Rheumatol 2012;64:1697–707.
2. Evans DS, Cailotto F, Parimi N, Valdes AM, Castaño-Betancourt MC, Liu Y, *et al*. Genome-wide association and functional studies identify a role for IGFBP3 in hip osteoarthritis. Ann Rheum Dis 2014 (in press).
3. Styrkarsdottir U, Thorleifsson G, Helgadottir HT, Bomer N, Metrustry S, Bierma-Zeinstra S, *et al*. Severe osteoarthritis of the hand associates with common variants within the ALDH1A2 gene and with rare variants at 1p31. Nat Genet 2014;46:498–502.
4. Evangelou E, Kerkhof HJ, Styrkarsdottir U, Evangelia NE, Bos SD, Esko T, *et al*. A meta-analysis of genome-wide association studies identifies novel variants associated with osteoarthritis of the hip. Ann Rheum Dis 2014;73:2130–6.
5. Evangelou E, Valdes AM, Castano-Betancourt MC, Doherty M, Doherty S, Esko T, *et al*. The DOT1L rs12982744 polymorphism is associated with osteoarthritis of the hip with genome-wide statistical significance in males. Ann Rheum Dis 2013;72:1264–5.
6. arcOGEN ConsortiumarcOGEN CollaboratorsZeggini E, Panoutsopoulou K, Southam L, Rayner NW, *et al*. Identification of new susceptibility loci for osteoarthritis (arcOGEN): a genome-wide association study. Lancet 2012;2012(380):815–23.
7. Day-Williams AG, Southam L, Panoutsopoulou K, Rayner NW, Esko T, Estrada K, *et al*. A variant in MCF2L is associated with osteoarthritis. Am J Hum Genet 2011;2011(89):446–50.
8. Panoutsopoulou K, Southam L, Elliott KS, Rayner NW, Zhai G, Beazley C, *et al*. Insights into the genetic architecture of osteoarthritis from stage 1 of the arcOGEN study. Ann Rheum Dis 2011;70:864–7.
9. Kerkhof HJM, Lories RJ, Meulenbelt I, Jonsdottir I, Valdes AM, Arp P, *et al*. A genome-wide association study identifies an osteoarthritis susceptibility locus on chromosome 7q22. Arthritis Rheumatol 2010;62:499–510.
10. Rodriguez-Fontenla C, Calaza M, Evangelou E, Valdes AM, Arden N, Blanco FJ, *et al*. Assessment of osteoarthritis candidate genes in a meta-analysis of 9 genome-wide association scans. Arthritis Rheumatol 2014;66:940–9.
11. Nakajima M, Takahashi A, Kou I, Rodriguez-Fontenla C, Gomez-Reino JJ, Furuichi T, *et al*. New sequence variants in HLA class II/III region associated with susceptibility to knee osteoarthritis identified by genome-wide association study. PLoS One 2010;5:e9723.
12. Shi D, Zheng Q, Chen D, Zhu L, Qin A, Fan J, *et al*. Association of single-nucleotide polymorphisms in HLA class II/III region with knee osteoarthritis. Osteoarthritis Cartilage 2010;18:1454–7.
13. Valdes AM, Styrkarsdottir U, Doherty M, Morris DL, Mangino M, Tamm A, *et al*. Large scale replication study of the association between HLA class II/BTNL2 variants and osteoarthritis of the knee in European-descent populations. PLoS One 2011;6:e23371.
14. Tsezou A. Osteoarthritis year in review 2014: genetics and genomics. Osteoarthritis Cartilage 2014;22:2017–24.
15. Im GI, Choi YJ. Epigenetics in osteoarthritis and its implication for future therapeutics. Expert Opin Biol Ther 2013;13:713–21.
16. Le LT, Swingler TE, Clark IM. The role of microRNAs in osteoarthritis and chondrogenesis. Arthritis Rheumatol 2013;65:1963–74.
17. Barter MJ, Bui C, Young DA. Epigenetic mechanisms in cartilage and osteoarthritis: DNA methylation, histone modifications and microRNAs. Osteoarthritis Cartilage 2012;20:339–49.
18. Young D, Bui C, Barter MJ. Understanding CpG methylation in the context of osteoarthritis. Epigenomics 2012;4:593–5.
19. Hashimoto K, Otero M, Imagawa K, de Andrés MC, Coico JM, Roach HI, *et al*. Regulated transcription of human matrix metalloproteinase 13 (MMP13) and interleukin-1β (IL1B) genes in chondrocytes depends on methylation of specific proximal promoter CpG sites. J Biol Chem 2013;288:10061–72.
20. Hashimoto K, Oreffo RO, Gibson MB, Goldring MB, Roach HI. DNA demethylation at specific CpG sites in the IL1B promoter in response to inflammatory cytokines in human articular chondrocytes. Arthritis Rheumatol 2009;60:3303–13.
21. Chabane N, Zayed N, Afif H, Mfuna-Endam L, Benderdour M, Boileau C, *et al*. Histone deacetylase inhibitors suppress interleukin-1β-induced nitric oxide and prostaglandin E2 production in human chondrocytes. Osteoarthritis Cartilage 2008;16:1267–74.
22. Culley KL, Hui W, Barter MJ, Davidson RK, Swingler TE, Destrument AP, *et al*. Class I histone deacetylase inhibition modulates metalloproteinase expression and blocks cytokine-induced cartilage degradation. Arthritis Rheumatol 2013;65:1822–30.
23. Miyaki S, Nakasa T, Otsuki S, Grogan SP, Higashiyama R, Inoue A, *et al*. MicroRNA-140 is expressed in differentiated human articular chondrocytes and modulates interleukin-1 responses. Arthritis Rheumatol 2009;60:2723–30.
24. Yang B, Kang X, Xing Y, Dou C, Kang F, Li J, *et al*. Effect of microRNA-145 on IL-1β-induced cartilage degradation in human chondrocytes. FEBS Lett 2014;588:2344–52.
25. Park SJ, Cheon EJ, Lee MH, Kim HA. MicroRNA-127-5p regulates matrix metalloproteinase 13 expression and interleukin-1β-induced catabolic effects in human chondrocytes. Arthritis Rheumatol 2013;65:3141–52.
26. Li J, Huang J, Dai L, Yu D, Chen Q, Zhang X, *et al*. miR-146a, an IL-1β responsive miRNA, induces vascular endothelial growth factor and chondrocyte apoptosis by targeting Smad4. Arthritis Res Ther 2012;14:R75.
27. Den Hollander W, Ramos YF, Bos SD, Bomer N, van der Breggen R, Lakenberg N, *et al*. Knee and hip articular cartilage have distinct epigenomic landscapes: implications for future cartilage regeneration approaches. Ann Rheum Dis 2014;73:2208–12.
28. Jeffries MA, Donica M, Baker LW, Stevenson ME, Annan AC, Humphrey MB, *et al*. Genome-wide DNA methylation study identifies significant epigenomic changes in osteoarthritic cartilage. Arthritis Rheumatol 2014;66:2804–15.
29. Moazedi-Fuerst FC, Hofner M, Gruber G, Weinhaeusel A, Stradner MH, Angerer H, *et al*. Epigenetic differences in human cartilage between mild and severe OA. J Orthop Res 2014;32:1636–45.
30. Rushton MD, Reynard LN, Barter MJ, Refaie R, Rankin KS, Young DA, *et al*. Characterization of the cartilage DNA methylome in knee and hip osteoarthritis. Arthritis Rheumatol 2014;66:2450–60.
31. Fernández-Tajes J, Soto-Hermida A, Vázquez-Mosquera ME, Cortés-Pereira E, Mosquera A, Fernández-Moreno M, *et al*. Genome-wide DNA methylation analysis of articular chondrocytes reveals a cluster of osteoarthritic patients. Ann Rheum Dis 2014;73:668–77.

32. Delgado-Calle J, Fernández AF, Sainz J, Zarrabeitia MT, Sañudo C, García-Renedo R, *et al.* Genome-wide profiling of bone reveals differentially methylated regions in osteoporosis and osteoarthritis. Arthritis Rheumatol 2013;65:197–205.
33. Hill CL, Gale DG, Chaisson CE, Skinner K, Kazis L, Gale ME, *et al.* Knee effusions, popliteal cysts, and synovial thickening: association with knee pain in osteoarthritis. J Rheumatol 2001;28:1330–7.
34. Hall M, Doherty S, Courtney P, Latief K, Zhang W, Doherty M. Synovial pathology detected on ultrasound correlates with the severity of radiographic knee osteoarthritis more than with symptoms. Osteoarthritis Cartilage 2014;22:1627–33.
35. Wang Q, Rozelle AL, Lepus CM, Scanzello CR, Song JJ, Larsen DM, *et al.* Identification of a central role for complement in osteoarthritis. Nat Med 2011;17:1674–9.
36. Sadouk MB, Pelletier JP, Tardif G, Kiansa K, Cloutier JM, Martel-Pelletier J. Human synovial fibroblasts coexpress IL-1 receptor type I and type II mRNA. The increased level of the IL-1 receptor in osteoarthritic cells is related to an increased level of the type I receptor. Lab Invest 1995;73:347–55.
37. Saas J, Haag J, Rueger D, Chubinskaya S, Sohler F, Zimmer R, *et al.* IL-1β, but not BMP-7 leads to a dramatic change in the gene expression pattern of human adult articular chondrocytes – portraying the gene expression pattern in two donors. Cytokine 2006;36:90–9.
38. Karlsson C, Dehne T, Lindahl A, Brittberg M, Pruss A, Sittinger M, *et al.* Genome-wide expression profiling reveals new candidate genes associated with osteoarthritis. Osteoarthritis Cartilage 2010;18:581–92.
39. Xu Y, Barter MJ, Swan DC, Rankin KS, Rowan AD, Santibanez-Koref M, *et al.* Identification of the pathogenic pathways in osteoarthritic hip cartilage: commonality and discord between hip and knee OA. Osteoarthritis Cartilage 2012;20:1029–38.
40. Attur M, Belitskaya-Lévy I, Oh C, Krasnokutsky S, Greenberg J, Samuels J, *et al.* Increased interleukin-1β gene expression in peripheral blood leukocytes is associated with increased pain and predicts risk for progression of symptomatic knee osteoarthritis. Arthritis Rheumatol 2011;63:1908–17.
41. Englund M, Guermazi A, Roemer F, Aliabadi P, Yang M, Lewis CE, *et al.* Meniscal tear in knees without surgery and the development of radiographic osteoarthritis among middle-aged and elderly persons. Arthritis Rheumatol 2009;60:831–9.
42. Scanzello CR, McKeon B, Swaim BH, DiCarlo E, Asomugha EU, Kanda V, *et al.* Synovial inflammation in patients undergoing arthroscopic meniscectomy: molecular characterization and relationship to symptoms. Arthritis Rheumatol 2011;63:391–400.
43. Tang C, Hsu C, Fong Y. The CCL5/CCR5 axis promotes interleukin-6 production in human synovial fibroblasts. Arthritis Rheumatol 2010;62:3615–24.
44. Vance D, Wang L, Rampersaud E, Lesniak PB, Vance JM, Pericak-Vance MA, *et al.* Dynamic gene expression profile changes in synovial fluid following meniscal injury; osteoarthritis (OA) Markers found. J Exerc Sports Orthop 2014;1:1–7.
45. Mobasheri A, Bondy CA, Moley K, Mendes AF, Rosa SC, Richardson SM, *et al.* Facilitative glucose transporters in articular chondrocytes. Expression, distribution and functional regulation of GLUT isoforms by hypoxia, hypoxia mimetics, growth factors and pro-inflammatory cytokines. Adv Anat Embryol Cell Biol 2008;200:1.
46. Scanzello CR, Umoh E, Pessler F, Diaz-Torne C, Miles T, Dicarlo E, *et al.* Local cytokine profiles in knee osteoarthritis: elevated synovial fluid interleukin-15 differentiates early from end-stage disease. Osteoarthritis Cartilage 2009;17:1040–8.
47. Snelling S, Rout R, Davidson R, Clark I, Carr A, Hulley PA, *et al.* A gene expression study of normal and damaged cartilage in anteromedial gonarthrosis, a phenotype of osteoarthritis. Osteoarthritis Cartilage 2014;22:334–43.
48. Ramos Y, den Hollander W, Bovée J, Bomer N, van der Breggen R, Lakenberg N, *et al.* Genes involved in the osteoarthritis process identified through genome wide expression analysis in articular cartilage; the RAAK study. Arthritis Rheumatol 2014;63:391–400.
49. Geyer M, Grässel S, Straub RH, Schett G, Dinser R, Grifka J, *et al.* Differential transcriptome analysis of intraarticular lesional vs intact cartilage reveals new candidate genes in osteoarthritis pathophysiology. Osteoarthritis Cartilage 2009;17:328–35.
50. Hoek RM, de Launay D, Kop EN, Yilmaz-Elis AS, Lin F, Reedquist KA, *et al.* Deletion of either CD55 or CD97 ameliorates arthritis in mouse models. Arthritis Rheumatol 2010;62:1036–42.
51. Lambert C, Dubuc JE, Montell E, Vergés J, Munaut C, Noël A, *et al.* Gene expression pattern of cells from inflamed and normal areas of osteoarthritis synovial membrane. Arthritis Rheumatol 2014;66:960–8.
52. Sato T, Konomi K, Yamasaki S, Aratani S, Tsuchimochi K, Yokouchi M, *et al.* Comparative analysis of gene expression profiles in intact and damaged regions of human osteoarthritic cartilage. Arthritis Rheumatol 2006;54:808–17.
53. Kuai J, Gregory B, Hill A, Pittman DD, Feldman JL, Brown T, *et al.* TREM-1 expression is increased in the synovium of rheumatoid arthritis patients and induces the expression of pro-inflammatory cytokines. Rheumatology (Oxford) 2009;48:1352–8.
54. Zhang W, Moskowitz RW, Nuki G, Abramson S, Altman RD, Arden N, *et al.* OARSI recommendations for the management of hip and knee osteoarthritis, Part II: OARSI evidence-based, expert consensus guidelines. Osteoarthritis Cartilage 2008;16:137–62.
55. Richy F, Bruyere O, Ethgen O, Rabenda V, Bouvenot G, Audran M, *et al.* Time dependent risk of gastrointestinal complications induced by non-steroidal anti-inflammatory drug use: a consensus statement using a meta-analytic approach. Ann Rheum Dis 2004;63:759–66.
56. Motsko SP, Rascati KL, Busti AJ, Wilson JP, Barner JC, Lawson KA, *et al.* Temporal relationship between use of NSAIDs, including selective COX-2 inhibitors, and cardiovascular risk. Drug Saf 2006;29:621–32.
57. Pincus T, Koch GG, Sokka T, Lefkowith J, Wolfe F, Jordan JM, *et al.* A randomized, double-blind, crossover clinical trial of diclofenac plus misoprostol versus acetaminophen in patients with osteoarthritis of the hip or knee. Arthritis Rheumatol 2001;14:1587–98.
58. Bradley JD, Katz BP, Brandt KD. Severity of knee pain does not predict a better response to an antiinflammatory dose of ibuprofen than to analgesic therapy in patients with osteoarthritis. J Rheumatol 2001;28:1073–6.
59. Beyer C, Zampetaki A, Lin NY, Kleyer A, Perricone C, Iagnocco A, *et al.* Signature of circulating microRNAs in osteoarthritis. Ann Rheum Dis 2015 (in press).
60. Ramos YF, Bos SD, Lakenberg N, Böhringer S, den Hollander WJ, Kloppenburg M, *et al.* Genes expressed in blood link osteoarthritis with apoptotic pathways. Ann Rheum Dis 2014;73:1844–53.